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Award Number: W81XWH-06-1-0400

TITLE:

Sonophoresis for Rapid Assessment of Interstitial Fluid and Drug Delivery

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REPORT DATE: October 2007

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE 01-oct-2007		2. REPORT TYPE Final		3. DATES COVERED 20 Apr 2006 - 29 Nov 2007	
4. TITLE AND SUBTITLE Sonophoresis for Rapid Assessment of Interstitial Fluid and Drug Delivery				5a. CONTRACT NUMBER W81XWH-06-1-0400	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Samir Mitragotri  Email: samir@engineering.ucsb.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of California Santa Barbara, CA 93106				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
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15. SUBJECT TERMS Ultrasonically, Keratin, Biomolecules					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
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## Introduction

The general objective of the proposed studies was to develop sonophoresis as a platform technology for assessing interstitial fluid from the skin and perform transdermal drug delivery. In this method, a short application of low-frequency ultrasound is used to extract biomarkers from skin. Ultrasound-permeabilized skin can also be used for transdermal drug delivery. Specifically, a drug patch can be placed on permeabilized skin to allow steady diffusion of drugs across the skin. Skin offers an excellent interface for diagnostics and drug delivery. However, low permeability of the skin limits our ability to harvest a sample of markers for diagnostics and to deliver drugs for therapeutic intervention. This challenge can be addressed by ultrasound. We proposed to accomplish four specific objectives:

- (i) Characterize the effect of ultrasound on skin ultrastructure and permeability. Through these studies we planned to establish the structural basis for enhanced skin permeability during sonophoresis.
- (ii) Characterize the chemical composition of the tissue fluid extracted by ultrasound. Through these studies we planned to establish the baseline composition of transdermally extracted fluid.
- (iii) Assess the immune response generated by transdermally delivered vaccines. Through these studies we planned to demonstrate the ability of sonophoresis to perform transcutaneous immunization.
- (iv) Evaluate the safety concerns associated with skin exposure to ultrasound. Through these studies we planned to establish safety of ultrasound under the operating conditions.

We made substantial progress in all four objectives. Achievement of milestones for each objective was discussed in quarterly reports. Here, we summarize the most significant accomplishment of the project as a whole, that is, establishment of a diagnostic platform.

## Body

Advances in analytical techniques have made a tremendous impact on diagnosis of various ailments including diabetes, cancer and cardiovascular diseases. Current diagnostic tools typically assay body fluids, primarily blood, for the presence of disease markers. However, many diseases have origins or manifestation locally in tissues. In such cases, methods based on tissue chemistry, instead of blood chemistry, can potentially provide accurate, sensitive and early diagnosis. Tissue chemistry, however, is rarely used in current diagnostic methods due to lack of easy-to-use, standardized methods for sample collection and analysis. Instead, tissue-based diagnosis is invariably performed using histology, which suffers from various limitations including its invasiveness and qualitative nature. We have developed a method that uses skin extract for quantitative and non-invasive diagnosis of disease conditions. Skin chemistry is not only a sensitive indicator of local diseases, for example skin cancer, psoriasis and eczema, but also of certain systemic diseases such as cardiovascular disease and diabetes. In addition, skin chemistry also holds information about body's exposure to exogenous chemical and biological entities<sup>1, 2</sup>. However, skin chemistry is not used in current diagnostic procedures due to lack of methods for harvesting skin constituents in a reliable way. A few methods that make use of ultrasound, electric current and microneedles have been tested for extracting systemic markers such as glucose from the skin<sup>3, 4</sup>; however, these methods have not been used to determine a comprehensive skin chemistry panel for diagnostic applications.

Our method employs a brief exposure of ultrasound (20 kHz) to skin to rapidly extract several types of structural and signaling proteins (keratins,  $\beta$ -actin, interleukins, chemokines, growth factors, antibodies and others), lipids (cholesterol, ceramides, fatty acids, triglycerides and others), nucleic acids (RNA and DNA), small-molecules (free amino acids, lactate and exogenous molecules) and microbes (bacteria), enabling us to create a unique biomolecular signature of the skin. All in all, ultrasound extracted at least a total of 71 proteins, 14 types of lipids, nucleic acids, 18 small-molecules as well as skin-resident bacteria from skin (Figure 1).

Ultrasonically extracted proteins from porcine skin exhibited high congruence with and homogenized epidermal proteins (Figure 1a, lanes 1,  $2237.3 \pm 101.7 \mu\text{g}/\text{cm}^2$  of saline-soluble and  $1871.7 \pm 62.7 \mu\text{g}/\text{cm}^2$  of insoluble protein was extracted by ultrasound). Western blot analysis of ultrasonically extracted proteins confirmed the presence of many structural proteins such as keratin filament subfamilies (K1, K5, K10, K14 and others) were detected in the skin extracts (figure 1b). This keratin profile matched with that found in full epidermis extracts. Notably, some of the insoluble higher molecular weight keratins that are chiefly expressed in the outermost layer – stratum corneum (SC) were present in the soluble fraction of the ultrasound extract (lane 1, figure 1b). Ultrasound also extracted several cytoplasmic proteins (heat-shock proteins: hsp60 (figure 1c) and hsp70 (figure 1d), and  $\beta$ -actin (figure 1e)) present in deeper nucleated layers of epidermis. Additional extraction experiments on human cadaver skin also revealed similar results.

Ultrasound also extracted 6 types of non-polar lipids (paraffin hydrocarbons (PH), squalene (SQ), cholesteryl esters (CE), triglycerides (TG), cholesteryl diesters (CD), and free fatty acids (FA); figure 1f) and 8 classes of polar lipids (cholesterol (CH), lanosterol (LA), and ceramide 1-6 (C1-6); figure 1g). A high resemblance of lipid profiles obtained from ultrasound extracts and the SC (figure 1f and 1g) was observed. Several other types of small-molecules including natural moisturizing factors (NMFs), 15 free amino acids (see Table A1.1 in the supplementary text), lactic acid ( $0.013 \pm 0.001 \text{ g/L}$ ), urea ( $0.148 \pm 0.054 \text{ mg/dL}$ ) and glucose ( $0.274 \pm 0.106 \text{ mg/dL}$ ) were also detected in the ultrasound extracts. In addition, nucleic acids (RNA and DNA,  $167.9 \pm 71.0 \text{ ng}/\text{cm}^2$ ) were also found in the extract, thus indicating that ultrasound retrieves biomolecular information from nucleated layers of skin. Surprisingly, ultrasound also extracted bacteria from the skin much more efficiently than those obtained by controls and the conventional cotton swabbing procedure (Figure 1h,  $p < 0.016$ ). Coagulase Negative Staphylococcus was found to be the dominant resident-bacterium on the analyzed porcine skin.

The ability of ultrasonically extracted biomolecules to sensitively reflect changes in skin's molecular composition was tested in three models; chemical-induced skin irritation (CI), atopic dermatitis (AD) and psoriasis (PS). While acetone-extracted mice provided a model for immediate inflammatory responses in the skin, atopic dermatitis and psoriasis mice models were used for analyzing sustained pathogenesis in the skin. A complete list of changes in biomolecular profile is provided in the supplementary text. Some of the key findings are presented here. Ultrasound extracted significantly higher amount of proteins from diseased mice as compared to healthy ones (AD – 2.32-fold and PS – 2.47-fold,  $p < 0.05$ ). Analysis of ultrasonic extracts from CI skin accurately demonstrated a relative decrease in the barrier-forming non-polar lipids of the SC (figure 2a, details in Figure SA2.1 of supplementary text). Up/down regulation of 62 inflammatory cytokines (interleukins, chemokines, colony stimulating factors, growth factors, and others) in CI skin was assessed to generate a unique cytokine “functionality-map” that precisely represents skin's specific diseased milieu (Fig. 2b-CI and Fig. 3a-AD and PS,

additional data in. Supplementary text table A2.1 (CI), A2.2 (AD) and table A2.3 (PS)). Owing to their immense importance in skin pathology as prominent immunostimulatory mediators<sup>5</sup>, we chose to profile cytokines as the most significant group of proteins extracted by ultrasound. Cytokines are known to act in a short-ranged fashion in tissues resulting in their rapid extracellular uptake and an extremely short half-life. Current biopsy-based techniques for cytokine detection are limited by either requiring a large protein sample or utilize *in situ* hybridization of mRNA, which doesn't necessarily reflect the presence or the actual quantity of the translated cytokine in the tissue<sup>6</sup>. Sensitive detection of several classes of these signaling molecules through ultrasonic extraction demonstrates the novelty and sensitivity of this diagnostic technique. A quantitative representation of each of the analyzed diseases yielded a unique cytokine "functionality map", which correlates with the specific diseased state (figure 2d and 3a). A striking, rapid upregulation was observed for some of these cytokines in acetone-challenged skin, while AD and PS exhibited different induction patterns.

Several antibodies were also found in diseased skin samples (Fig. 3c). Consistent with the presence of immuno-deposits in autoimmune diseases, particularly in psoriasis,<sup>7</sup> an increased amount of IgG was extracted from AD (7.74-fold,  $p < 0.05$ ) and PS (2.15-fold,  $p < 0.05$ ) skin. However, IgE was only detected in ultrasonic extracts from AD skin, which is also consistent with a strong association of these antibodies with epidermal immune cells in AD<sup>8</sup>.

Specific quantitative lipid profiles for these diseases were also constructed (supplementary text table A3.1). AD and PS mice revealed significant alterations in the epidermal lipid composition (Fig. 3b, healthy (lane C), AD (lane AD) and PS (lane PS) skin). Both AD and PS skin extracts showed a significant reduction in non-polar lipids (SQ and CE;  $p < 0.05$ ) and simultaneous upregulation of a few polar lipids (specifically, LA and CH in PS skin;  $p < 0.05$ ). Compared to healthy mice, increased amounts of nucleic acids (10-fold in RNA and 30-fold in DNA mass) were also observed. Abnormality in the relatively non-polar SC lipid barrier is an excellent indicator of several skin pathologies<sup>9</sup>. Ultrasound-assisted quantitative lipid profiling of both AD and PS skin extracts showed disease-specific and significant reduction in the non-polar lipids (SQ and CE), while a significant increase in some relatively polar lipid types was observed (figure 2a-c and 3b). Further, the total lipid extracted from the diseased skin was lower than the lipid content of normal skin. This verifies that both of these diseases have chronically reduced hydrophobicity and a compromised SC lipid barrier<sup>10, 11 12</sup>.

As another test, ultrasonic extraction procedure was tested in rats for detection of cocaine, a drug-of-abuse which is difficult to detect once it has been eliminated from the body through urine. Radiolabeled cocaine was administered to rats in a single injection. While cocaine levels rapidly fell to less than 0.25% in urine, ultrasonic extraction detected a sustained presence of cocaine in skin for up to 7 days after drug administration (Fig. 4b). This technique significantly improves upon the conventional urine-based diagnostics, where the drug is undetectable after a few days of intake<sup>13</sup>. The method also advances over the time-intensive sweat-collection patches, which have to be worn continuously for 7-10 days and are thereby subjected to be tampered by the drug-abuse subjects<sup>13</sup>.

The ability of ultrasound to monitor skin biomolecular composition can be utilized to detect not only abused drugs but also therapeutic drugs. This need arises from the fact that there are currently no approved techniques by the FDA for accurately evaluating drug pharmacokinetics in the skin<sup>14</sup>. Transdermal extraction of intravenously administered fluconazole, a model drug, was performed *in vivo* in Sprague Dawley rats. While, a short half-life of fluconazole in blood ( $5.79 \pm 2.76$  hr) was recorded, ultrasonic extraction revealed a

significantly higher half-life of  $92.82 \pm 12.83$  hr ( $p < 0.005$ ) in the skin. Skin swabs did not detect any substantial amount of drug in the skin. Ultrasound not only extracted drugs for extended periods of time but was more sensitive than tape stripping procedure<sup>15-17</sup>.

Safety of ultrasound under the conditions used in this study has been previously established *in vivo*<sup>18,19</sup>. Specifically, histology studies on skin exposed to ultrasound revealed no microscopic alterations in its structure<sup>20</sup>. Ultrasound-assisted skin profiling offers a novel approach for diagnostics. Non-invasive diagnostics based on molecular expression patterns offers numerous advantages and applications in varied fields such as dermatology, environmental exposure assessment and drug testing. This technique can be easily extended to other body surfaces such as mucosal membranes and with additional medications of the ultrasound device to other difficult-to-reach tissues.

### **Key Research Accomplishments**

- Establishment of ultrasound as a means of extracting skin constituents
- Establishment of chemistry of extract
- Confirmation of presence of specific proteins/lipids in the extract
- Validation of extraction for model pathological conditions

### **Reportable outcomes**

- Manuscript under preparation

### **Conclusions**

Our project successfully showed extraction of various important biomarkers from skin using ultrasound. The biomolecular composition of human skin and mucosal membrane consists of multitude of lipids, proteins, nucleic acids and other miscellaneous molecules. Skin's molecular composition is a sensitive indicator of its local health as well as body's systemic health. However, this information is not easily accessible and consequently not used in the current diagnostic methods, which are based on visual observations and/or biopsy. The severe lack of accurate methods to retrieve biomolecular information from the skin is in stark contrast with the wealth of methods that have been developed to deliver molecules into skin. Some of these methods, for example iontophoresis and ultrasound, have been utilized for detecting blood chemistry (glucose in particular). However, currently no methods exist to quantitatively determine skin's biomolecular profile for diagnostic applications. We have developed a method that directly addresses this critical unmet need. We rapidly and non-invasively extract biological markers from the stratum corneum and epidermis. We extracted several types of structural and signaling proteins (keratins,  $\beta$ -actin, interleukins, chemokines, antibodies, growth factors and others), lipids (cholesterol, ceramides, fatty acids, triglycerides and others), nucleic acids (RNA and DNA), small-molecules (free amino acids, lactate and exogenous molecules) and microbes (bacteria) enabling us to create skin's unique "biomolecular signature". The extracted biomolecules showed quantitative correlations with their composition in the stratum corneum and epidermis. Extraction of these entities from skin using our method is convenient and reproducible, thus making it an advantageous alternative to biopsy and tape-stripping. By quantifying changes in skin's biomolecular profile, we have demonstrated several specific *in vivo* applications for assessing chemical-induced skin irritation, atopic dermatitis and psoriasis in animal models. Our skin profiling method offers a novel, non-invasive and quantitatively sensitive technique which has numerous applications in varied fields such as dermatology, cancer diagnostics, exposure/risk assessment, and drug testing.

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## FIGURE CAPTIONS

**FIGURE 1:** Construction of the molecular signature of skin. The ability of ultrasound to quantitatively extract several epidermal biomolecules including proteins (a-g), lipids (h-i), and bacteria (j) was assessed *in vitro* in porcine skin. (a) SDS PAGE analysis revealed a high congruence between the protein migration patterns of the ultrasonically extracted (lane 2) and the native proteins expressed in the epidermal skin (lane 1). (c-d) Western blot analysis of the saline-insoluble ultrasound extracts from porcine skin (lane 2 in fig. c) and from human skin (lane 1 in fig. d) showed the specific presence of many keratin filament subfamilies (K1, K5, K10, K14, K19 and others). These keratin profiles matched with those found in the respective full epidermis extracts (lane 3 in fig. c; lane 2 in fig. d). Some of the insoluble and higher molecular weight keratins were also present in the saline-soluble fraction of the porcine ultrasound extract (lane 1 in fig. c). (e-g) Ultrasound (lane1) also extracted several cytoplasmic proteins from the viable epidermis (heat-shock proteins: hsp60 (e) and hsp70 (f), and  $\beta$ -actin (g)). Lane 2 verifies the presence of these proteins in porcine epidermis (h-i) Thin-layer chromatographs of non-polar lipids (h) and polar lipids (i) demonstrated a high resemblance in lipid profiles obtained from porcine ultrasound extracts (US) and lipids isolated from stratum corneum (SC). *PH*: Paraffin hydrocarbons; *SQ*: Squalene; *CE*: Cholesteryl esters; *TG*: Triglycerides; *CD*: Cholesteryl diesters; *FA*: Free fatty acids; *LA*: Lanosterol; *CH*: Cholesterol; *CI-6*: Ceramide 1-6; *OP*: Other polar lipids (glucosyl-derivates and phospholipids); (\*): Sodium lauryl sulfate. (j) Ultrasound efficiently extracted bacteria from porcine skin measured by a significantly higher colony-forming unit (CFU) than obtained from either the conventional cotton swabbing procedure or simple incubation of skin with saline. All data are representative of a minimum of 3 samples.

**FIGURE 2:** Quantitative assessment of acetone-induced acute barrier disruption of skin. (a) Gravimetric analysis of ultrasonic extracts from mice skin treated with acetone revealed a significant decrease in the total lipid (1.35-fold) as well as the non-polar lipids (1.65-fold) of the acetone-disrupted skin. (b) Lipid chromatography further suggested that acetone (AE) exclusively extracts non-polar lipids from mice skin. Acetone extracted lipids were present in reduced amounts in the acetone-treated skin extract (A) as compared to normal skin extract (C). *MO*: Methyl oleate; *UN1*: Unknown-1. (c) *MO* and *UN1* lipids were chiefly affected by acetone treatment. (d) Ultrasonic extraction quantitatively captured the cytokine profiles expressed in acetone-treated skin. A “functionality map” consisting of 24 inflammatory cytokines was constructed by categorizing these proteins according to their functionality into 5 groups: interleukins, growth factors, colony stimulating factors, chemokines and others. A significant upregulation of several key cytokines in the acetone-treated skin was seen. All data are representative of a minimum of 3 samples.

**FIGURE 3:** Ultrasound-assisted diagnostics of diseased mice with atopic dermatitis or psoriasis. (a) Cytokine “functionality maps”, as described in figure 2, showed unique and disease-specific expression of 62 different signaling proteins in mice with atopic dermatitis (AD) or psoriasis (PS). Expression profile in each cytokine category was denoted in the map as: (# downregulated) # Upregulated / # Total analyzed. (b) Ultrasonic lipid extracts from AD and PS skin were quantitatively analyzed with lipid chromatography and significant alterations in the epidermal lipid composition were found when compared with healthy mice skin (C). Both AD and PS skin lipids showed a significant reduction in non-polar lipids (*SQ* and *CE*) and simultaneous upregulation of a few polar lipids (specifically, *LA* and *CH* in PS skin). (c) Immunoblots specific for IgG antibodies revealed that ultrasound extracted significantly higher amounts of immunoglobulin from diseased (AD and PS) skin as compared to healthy (C) skin. All data are representative of a minimum of 3 samples.

**FIGURE 4:** Ultrasonic extraction for assessing drug bioavailability and forensic detection of drugs-of-abuse in skin. **(a)** In comparison with the conventional sequential tape stripping procedure (25 times), ultrasound significantly increased the extraction of topically delivered behinol in porcine skin *in vitro*. **(b)** Behinol was delivered from the dermis-side of the skin for 8 or 24 hr to mimic the drug's systemic administration *in vitro*. Ultrasound method was more sensitive than tape stripping (25 times) in its ability to statistically distinguish between drugs extracted from porcine skin at the two different times. **(c)** Bioavailability of intravenously administrated fluconazole was assessed *in vivo*. While, blood level of the drug rapidly fell to a negligible amount within 24 hr, ultrasonic extraction revealed a significantly higher tissue retention-time of fluconazole in the skin. Fluconazole's skin bioavailability was successfully assessed by ultrasonic extraction for over 7 days, which was also confirmed by cyanoacrylate-tape stripping. Occasionally ultrasound extracted significantly higher amount of fluconazole than by tape stripping (indicated by (\*):  $p < 0.05$ , student's t-test). Skin swabs did not detect any substantial amount of drug in the skin. **(d)** Ultrasound-assisted forensic detection of intravenously delivered cocaine (as a model for drug-of-abuse) was tested in rats. Cocaine levels rapidly fell to less than 0.25% in urine; however, ultrasonic extraction detected a sustained presence of cocaine in skin for up to 7 days (\* indicates  $p < 0.05$  for student's t-test: urine vs. skin drug amount). Ultrasound technique presents a novel method that improves upon the conventional urine-based diagnostics and time-intensive sweat-collection patches. Error bars denote at least 3 data points.

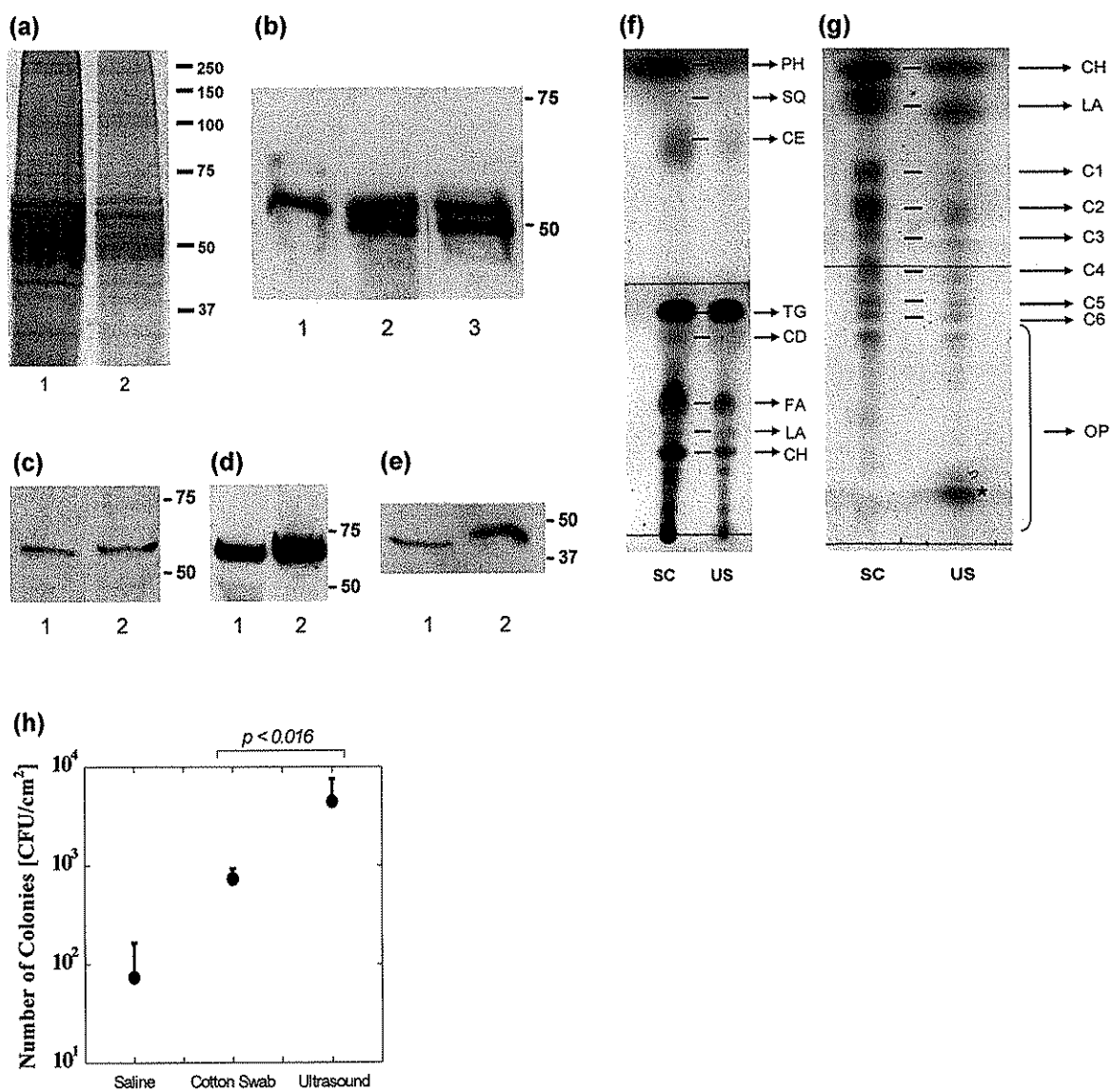
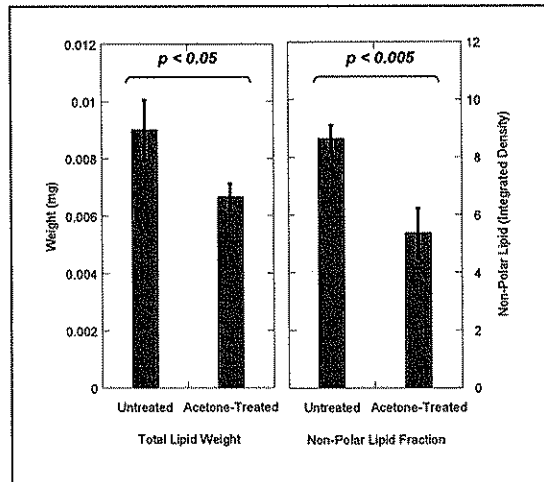


Figure 1

(a)



(b)

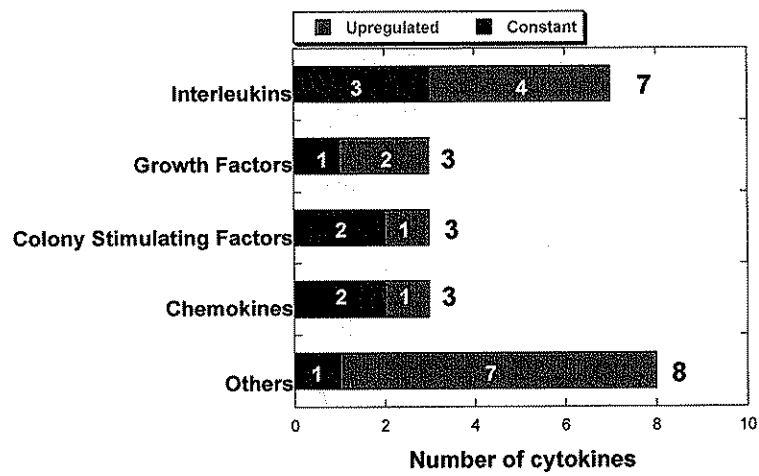


Figure 2

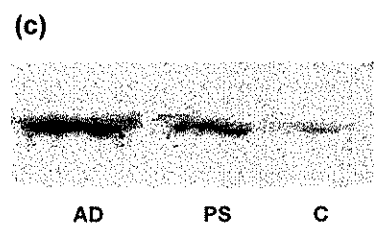
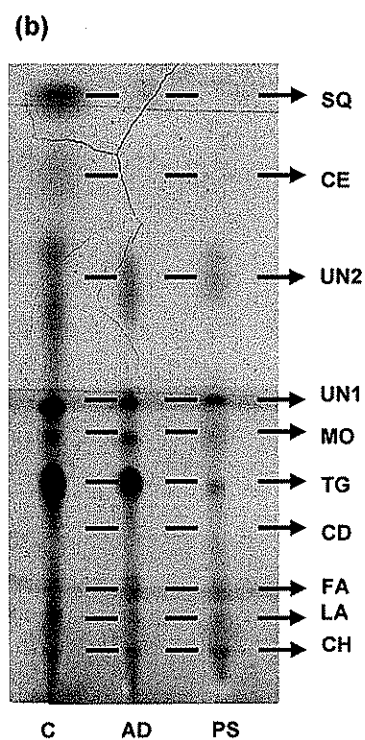
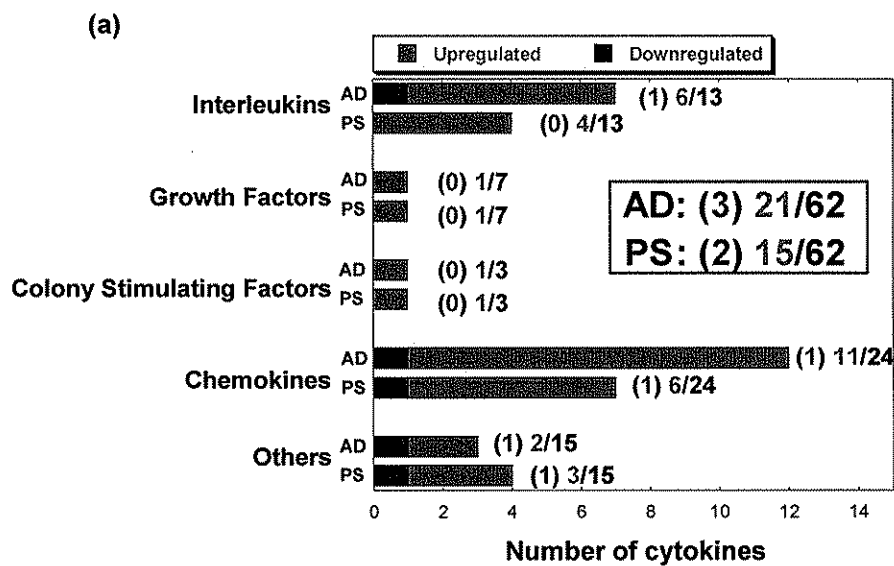


Figure 3

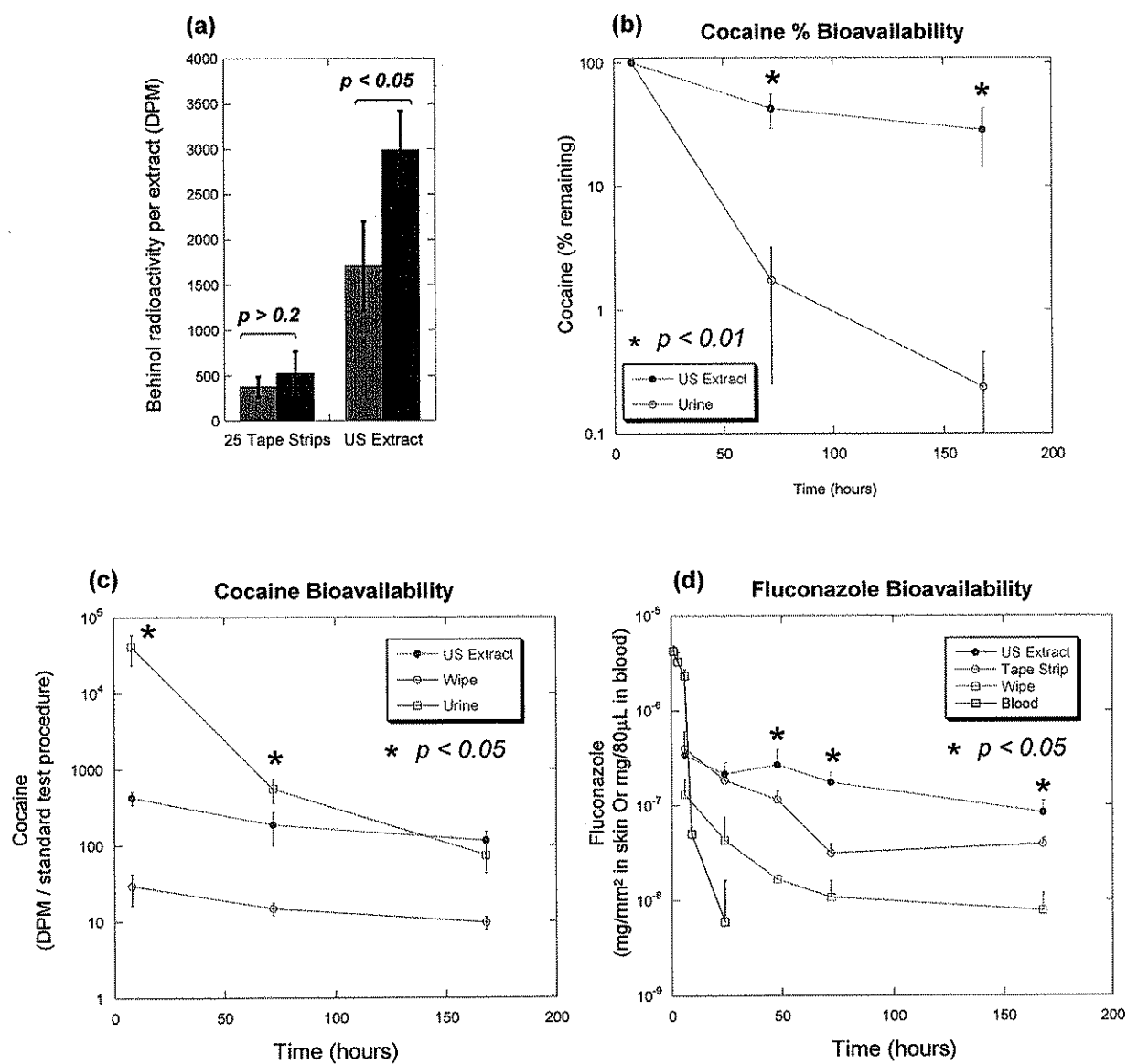
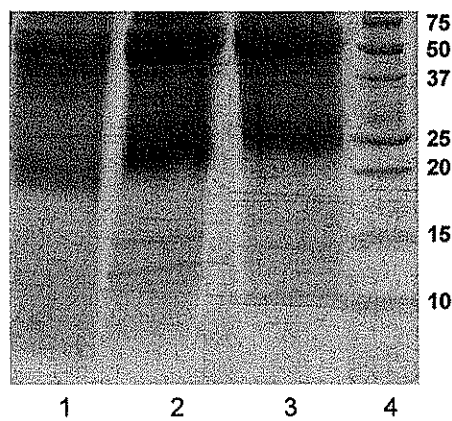


Figure 4

(a)



(b)

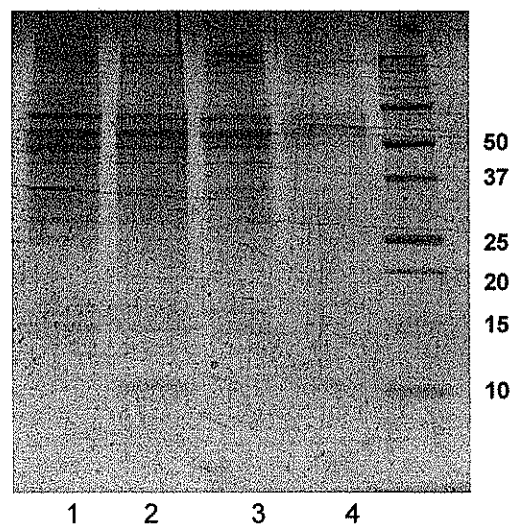


Figure A1

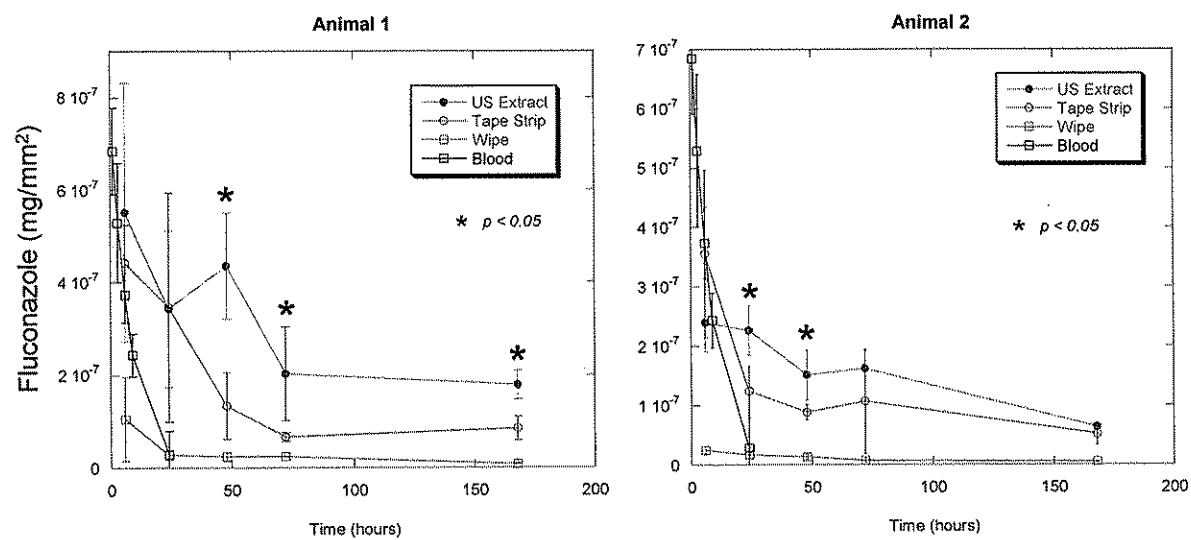


Figure A2

**Table A1.1 Concentration of Proteins and Nucleic acids in the extracts**

Animal Strain	Protein ( $\mu\text{g}/\text{cm}^2$ )		Nucleic acid ( $\text{ng}/\text{cm}^2$ )	
	Insoluble	Soluble	DNA	RNA
<i>In Vitro</i>				
Porcine skin	1871.7 $\pm$ 62.7	237.3 $\pm$ 101.7	43.5 $\pm$ 38.5	167.9 $\pm$ 71.0
Human skin	1536.7 $\pm$ 118.6	406.8 $\pm$ 220.1	nd	
<i>Animal Models (In Vivo)</i>				
Healthy Hairless Mice	431.3 $\pm$ 52.4	51.8 $\pm$ 1.9		nd
Acetone-treated Mice	495.1 $\pm$ 106.4	68.0 $\pm$ 20.6		nd
Healthy Mice (Balb/c)	641.8 $\pm$ 23.6	28.6 $\pm$ 4.0	0.7 $\pm$ 0.7	17.2 $\pm$ 1.7
Psoriasis Mice	1595.9 $\pm$ 85.0 *	60.7 $\pm$ 3.3 *	18.9 $\pm$ 25.1	167.8 $\pm$ 31.0 *
Atopic Dermatitis Mice	1485.2 $\pm$ 458.8 *	67.2 $\pm$ 15.4 *	14.2 $\pm$ 19.6	123.0 $\pm$ 122.3

(*nd*: not determined; \* represents a value of  $p < 0.05$  in student's t test)

**Table A1.2 Natural Moisturizing Factors: Free amino acid profile**

Amino Acid	Ultrasound Extract (%)	Stratum Corneum (%)	
Hydroxy praline (hyp)	$33.86 \pm 12.75$	$28.98 \pm 3.82$	*
Aspartic acid (asp)	$20.60 \pm 4.68$	$16.83 \pm 1.81$	*
Serine (ser)	$10.67 \pm 4.13$	$14.33 \pm 1.37$	*
Glutamic acid (glu)	$1.28 \pm 0.56$	$2.61 \pm 0.22$	
Proline (pro)	$2.66 \pm 1.44$	$3.62 \pm 0.42$	*
Glycine (gly)	$5.74 \pm 2.05$	$5.94 \pm 0.63$	*
Alanine (ala)	$3.67 \pm 1.33$	$3.30 \pm 0.33$	*
Cysteine (cys)	$0.70 \pm 0.41$	$1.06 \pm 0.11$	*
Isoleucine (ile)	$0.45 \pm 0.18$	$0.54 \pm 0.06$	*
Leucine (leu)	$0.90 \pm 0.34$	$0.89 \pm 0.12$	*
Tyrosine (tyr)	$0.53 \pm 0.26$	$0.71 \pm 0.08$	*
Phenylalanine (phe)	$0.52 \pm 0.22$	$0.68 \pm 0.07$	*
Histidine (his)	$2.37 \pm 0.83$	$5.08 \pm 0.45$	
Lysine (lys)	$0.86 \pm 0.36$	$0.89 \pm 0.11$	*
Arginine (arg)	$2.25 \pm 0.64$	$3.66 \pm 0.29$	

(\* represents a value of  $p > 0.3$  in student's t test)

**Table A2.1 Acute Barrier Disruption mouse model: Cytokine profile**

Interleukins				Growth Factors			
Name	Enhancement	p value		Name	Enhancement	p value	
IL-1b	2.86	0.043	**	Leptin	48.35	0.010	**
IL-9	2.28	0.032	**	IGF-II	3.06	0.042	**
IL-1a	1.80	0.002	**	bFGF	2.78	0.014	**
IL-12 p70	1.65	0.047	**	TPO	1.88	0.017	**
IL-6	1.76	0.093	*	VEGF	3.24	0.109	
IL-12 p40/p70	1.36	0.081	*				
IL-13	1.26	0.140					
Chemokines				Others			
Name	Enhancement	p value		Name	Enhancement	p value	
MCP1	2.77	0.015	**	IFNg	4.19	0.004	**
PF-4	1.86	0.005	**	TIMP-1	2.94	0.006	**
MIG	14.30	0.058	*	Fas ligand	1.83	0.032	**
Eotaxin	2.18	0.161		TNFa	1.62	0.031	**
				TIMP-2	1.55	0.067	*
Colony Stimulating Factors							
Name	Enhancement	p value					
GM-CSF	1.72	0.041	**				
M-CSF	2.02	0.067	*				
G-CSF	1.33	0.114					

(\*\* and \* represents a value of  $p < 0.05$  and  $p < 0.1$  in student's t test respectively)

**Table A2.2 Atopic Dermatitis mouse model: Cytokine profile**

Interleukins			
Name	Enhancement	p value	
IL-4	3.09	0.005	**
IL-6	3.03	0.027	**
IL-3	2.41	0.000	**
IL-1a	1.92	0.021	**
IL-5	1.65	0.005	**
IL-9	1.64	0.023	**
IL-1b	1.70	0.098	*
IL-12 p40/p70	1.61	0.384	
IL-2	1.54	0.190	
IL-13	1.21	0.504	
IL-12 p70	1.15	0.378	
IL-10	1.12	0.764	
IL-17	0.08	0.014	**

Colony Stimulating Factors			
Name	Enhancement	p value	
M-CSF	1.36	0.006	**
GM-CFS	1.75	0.183	
G-CSF	1.67	0.166	

Growth Factors			
Name	Enhancement	p value	
IGFBP-3	3.12	0.000	**
IGFBP-5	1.76	0.722	
TPO	1.61	0.217	
IGFBP-6	1.35	0.253	
VEGF	0.59	0.698	
Leptin	0.26	0.113	

Chemokines			
Name	Enhancement	p value	
TCA-3	2.45	0.004	**
TARC	2.35	0.004	**
TECK	2.23	0.000	**
CRG-2	1.75	0.003	**
RANTES	1.70	0.008	**
CTACK	1.68	0.000	**
Eotaxin-2	1.61	0.032	**
CXCL 16	1.60	0.005	**
MIP-1a	1.34	0.009	**
MCP1	1.34	0.016	**
MIP-2	1.22	0.038	**
MIP-1g	1.66	0.099	*
PF-4	1.44	0.064	*
MIP-3a	1.27	0.094	*
Fractalkine	1.72	0.210	
LIX	1.71	0.066	
Lymphotactin	1.26	0.403	
BLC	1.09	0.585	
MIP-3 b	1.06	0.925	
SDF-1a	0.68	0.635	
KC	0.68	0.598	
MIG	0.41	0.175	
MCP-5	0.42	0.047	**

Others			
Name	Enhancement	p value	
IL-3Rb	2.22	0.001	**
Leptin R	2.07	0.012	**
CD30L	2.06	0.054	*
CD30 T	1.69	0.094	*
P-selectin	1.57	0.052	*
sTNF RII	4.53	0.497	
sTNF R1	3.02	0.149	
Fas ligand	2.08	0.060	
L-selectin	1.52	0.169	
Axl	1.36	0.550	
VCAM-1	1.29	0.194	
CD40	1.12	0.769	
TNFA	1.09	0.492	
IFNg	0.94	0.830	
TIMP-1	0.16	0.006	**

(\*\* and \* represents a value of  $p < 0.05$  and  $p < 0.1$  in student's t test respectively)

**Table A2.3 Psoriasis mouse model: Cytokine profile**

Interleukins			
Name	Enhancement	p value	
IL-4	2.50	0.006	**
IL-3	1.98	0.002	**
IL-1a	1.35	0.019	**
IL-9	1.19	0.038	**
IL-5	1.69	0.098	*
IL-6	2.35	0.115	
IL-10	1.51	0.354	
IL-1b	1.14	0.486	
IL-12 p70	1.12	0.185	
IL-2	0.99	0.963	
IL-13	0.94	0.775	
IL-12 p40/p70	0.84	0.720	
IL-17	0.34	0.155	

Colony Stimulating Factors			
Name	Enhancement	p value	
G-CSF	1.31	0.029	**
M-CSF	1.10	0.380	
GM-CSF	1.08	0.826	

Growth Factors			
Name	Enhancement	p value	
IGFBP-3	2.79	0.000	**
IGFBP-6	1.21	0.072	*
IGFBP-5	10.30	0.354	
VEGF	4.83	0.342	
TPO	0.98	0.910	
Leptin	0.21	0.094	*

Chemokines			
Name	Enhancement	p value	
TECK	1.84	0.047	**
CRG-2	1.57	0.014	**
LIX	1.53	0.001	**
MIP-3a	1.36	0.004	**
CTACK	1.35	0.010	**
RANTES	1.34	0.006	**
TCA-3	1.75	0.059	*
MIP-1a	1.40	0.166	
Fractalkine	1.39	0.294	
MIP-1g	1.37	0.160	
TARC	1.14	0.343	
CXCL 16	1.07	0.650	
Eotaxin-2	1.06	0.595	
MCP1	1.04	0.738	
BLC	0.96	0.759	
MIP-3 b	0.91	0.707	
PF-4	0.91	0.253	
MIG	0.73	0.739	
KC	0.72	0.649	
Lymphotactin	0.72	0.202	
MIP-2	0.86	0.096	*
SDF-1a	0.00	0.071	*
MCP-5	0.04	0.000	**

Others			
Name	Enhancement	p value	
CD30L	1.72	0.031	**
IL-3Rb	1.57	0.044	**
Leptin R	1.49	0.003	**
Fas ligand	2.59	0.093	*
L-selectin	1.35	0.056	*
IFNg	1.99	0.222	
Axl	1.60	0.373	
TNFA	1.24	0.481	
VCAM-1	1.04	0.825	
P-selectin	1.02	0.928	
CD30 T	1.01	0.903	
CD40	0.66	0.439	
sTNF R1	0.63	0.490	
sTNF RII	0.00	0.391	
TIMP-1	0.00	0.002	**

(\*\* and \* represents a value of  $p < 0.05$  and  $p < 0.1$  in student's t test respectively)

**Table A3.1 Lipid profile: Animal disease models**

Lipids	Atopic Dermatitis		Psoriasis	
	Enhancement		Enhancement	
Squalene	0.41	*	0.37	*
Cholesteryl esters	0.32	*	0.27	*
UN2	0.64	*	0.63	*
UN1	1.04		1.38	*
Methyl Oleate	1.22		1.32	
Triglycerides	3.47		1.76	
Cholesteryl diesters	1.46		1.59	
Free fatty acids	1.21		1.55	
Lanosterol	0.96		1.30	*
Cholesterol	1.52		2.48	*
Polar Lipids	1.45	*	1.49	*

(\* represents a value of  $p < 0.05$  in student's t test)